**Supplemental Table 1.** Analytical and targeted amino acid (AA) concentration (% of the total AA) in some of the experimental diets used to feed rainbow trout fry\*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | LPLE1 | | LPLE9 | | LPME1 | | LPME9 | | LPHE1 | | LPHE9 | |
| % of the total AA | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value |
| L-Arginine | 6.54 | 6.46 | 6.88 | 6.40 | 6.27 | 6.46 | 6.91 | 6.40 | 6.59 | 6.46 | 6.91 | 6.40 |
| L-Histidine | 2.96 | 2.98 | 3.11 | 2.96 | 2.89 | 2.98 | 2.98 | 2.96 | 2.97 | 2.98 | 3.07 | 2.96 |
| L-Isoleucine | 4.39 | 4.38 | 4.35 | 4.33 | 4.70 | 4.38 | 4.71 | 4.33 | 4.29 | 4.38 | 4.17 | 4.33 |
| L-Leucine | 8.02 | 7.65 | 7.40 | 7.58 | 9.19 | 7.65 | 9.16 | 7.58 | 8.16 | 7.65 | 7.45 | 7.58 |
| **L-Lysine** | **0.94** | **0.91** | **15.17** | **14.38** | **0.95** | **0.91** | **14.47** | **14.38** | **0.98** | **0.91** | **15.87** | **14.38** |
| L-Methionine | 2.96 | 2.90 | 2.85 | 2.88 | 2.93 | 2.90 | 2.85 | 2.88 | 3.06 | 2.90 | 2.74 | 2.88 |
| L-Cystine | 1.12 | 1.18 | 1.19 | 1.17 | 1.20 | 1.18 | 1.25 | 1.17 | 1.19 | 1.18 | 1.31 | 1.17 |
| L-Phenylalanine | 4.48 | 4.42 | 4.61 | 4.37 | 4.53 | 4.42 | 4.71 | 4.37 | 4.42 | 4.42 | 4.63 | 4.37 |
| L-Threonine | 4.84 | 4.80 | 5.23 | 4.75 | 4.70 | 4.80 | 4.88 | 4.75 | 5.01 | 4.80 | 5.22 | 4.75 |
| L-Valine | 5.24 | 5.13 | 5.54 | 5.08 | 5.44 | 5.13 | 5.70 | 5.08 | 5.35 | 5.13 | 5.52 | 5.08 |
| L-Aspartic acid | 10.84 | 10.02 | 4.71 | 1.80 | 10.51 | 10.02 | 4.49 | 1.80 | 11.30 | 10.02 | 4.67 | 1.80 |
| L-Glutamic acid | 21.28 | 18.37 | 21.27 | 18.20 | 20.94 | 18.37 | 21.04 | 18.20 | 20.44 | 18.37 | 20.93 | 18.20 |
| L-Alanine | 6.81 | 6.62 | 3.11 | 2.83 | 6.51 | 6.62 | 3.02 | 2.83 | 6.93 | 6.62 | 3.16 | 2.83 |
| L-Glycine | 8.02 | 7.82 | 4.35 | 4.04 | 7.71 | 7.82 | 4.23 | 4.04 | 8.16 | 7.82 | 4.55 | 4.04 |
| L-Proline | 7.08 | 6.75 | 7.45 | 6.69 | 6.92 | 6.75 | 6.91 | 6.69 | 6.59 | 6.75 | 7.12 | 6.69 |
| L-Serine | 4.57 | 4.70 | 2.85 | 2.55 | 4.58 | 4.70 | 2.76 | 2.55 | 4.63 | 4.70 | 2.74 | 2.55 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | HPLE1 | | HPLE9 | | HPME1 | | HPME9 | | HPHE1 | | HPHE9 | |
| % of the total AA | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value | Analytical values | Targeted value |
| L-Arginine | 6.57 | 6.42 | 6.71 | 6.42 | 6.40 | 6.42 | 6.41 | 6.42 | 6.33 | 6.42 | 6.61 | 6.42 |
| L-Histidine | 2.98 | 2.97 | 3.04 | 2.97 | 2.93 | 2.97 | 2.91 | 2.97 | 2.99 | 2.97 | 2.96 | 2.97 |
| L-Isoleucine | 4.37 | 4.35 | 4.39 | 4.35 | 4.67 | 4.35 | 5.18 | 4.35 | 4.98 | 4.35 | 4.84 | 4.35 |
| L-Leucine | 7.53 | 7.61 | 7.54 | 7.61 | 7.58 | 7.61 | 7.56 | 7.61 | 7.29 | 7.61 | 7.71 | 7.61 |
| **L-Lysine** | **0.98** | **0.92** | **6.80** | **6.56** | **1.00** | **0.92** | **6.37** | **6.56** | **0.96** | **0.92** | **6.55** | **6.56** |
| L-Methionine | 2.86 | 2.89 | 2.78 | 2.89 | 2.90 | 2.89 | 2.89 | 2.89 | 2.81 | 2.89 | 2.82 | 2.89 |
| L-Cystine | 1.14 | 1.20 | 1.25 | 1.20 | 1.24 | 1.20 | 1.23 | 1.20 | 1.19 | 1.20 | 1.24 | 1.20 |
| L-Phenylalanine | 4.53 | 4.39 | 4.59 | 4.39 | 4.65 | 4.39 | 4.65 | 4.39 | 4.58 | 4.39 | 4.71 | 4.39 |
| L-Threonine | 4.98 | 4.77 | 4.99 | 4.77 | 4.83 | 4.77 | 4.88 | 4.77 | 4.78 | 4.77 | 4.98 | 4.77 |
| L-Valine | 5.37 | 5.10 | 5.35 | 5.10 | 5.35 | 5.10 | 5.74 | 5.10 | 5.47 | 5.10 | 5.55 | 5.10 |
| L-Aspartic acid | 10.67 | 9.96 | 8.07 | 6.43 | 10.63 | 9.96 | 7.97 | 6.43 | 10.65 | 9.96 | 8.06 | 6.43 |
| L-Glutamic acid | 21.56 | 18.63 | 22.38 | 18.63 | 21.59 | 18.63 | 21.71 | 18.63 | 21.62 | 18.63 | 22.02 | 18.63 |
| L-Alanine | 6.81 | 6.58 | 5.23 | 5.02 | 6.62 | 6.58 | 5.10 | 5.02 | 6.63 | 6.58 | 5.22 | 5.02 |
| L-Glycine | 7.83 | 7.78 | 6.42 | 6.22 | 7.80 | 7.78 | 6.31 | 6.22 | 7.80 | 7.78 | 6.45 | 6.22 |
| L-Proline | 7.10 | 6.84 | 6.86 | 6.84 | 7.12 | 6.84 | 7.52 | 6.84 | 7.29 | 6.84 | 6.63 | 6.84 |
| L-Serine | 4.69 | 4.67 | 3.63 | 3.36 | 4.67 | 4.67 | 3.59 | 3.36 | 4.70 | 4.67 | 3.69 | 3.36 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |

\* Tyrosine and tryptophan could not be determined with the amino acid analysis method used

**Supplemental Table 2.** Body weight gain, thermal growth coefficient (TGC; 1000 x ((final body weight)1/3 – (initial body weight)1/3)/(feeding days x temperature), feed efficiency (FE; wet weight gain/DM intake) and protein efficiency ratio (PER; wet weight gain/DP intake) of rainbow trout fry fed 81 diets containing three digestible protein (DP) levels and three digestible energy (DE) levels supplemented with nine graded levels of L-lysine (Lys).HCl for 24 (MP diets) or 30 (LP and HP diets) feeding days\*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diet no. |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Dietary Lys concentration (g/kg DM) | LP | 2.26 | 5.38 | 8.51 | 11.63 | 14.75 | 17.88 | 21.00 | 28.50 | 36.00 |
|  | MP | 3.67 | 7.71 | 11.75 | 15.79 | 19.83 | 23.87 | 27.92 | 31.96 | 36.00 |
|  | HP | 5.07 | 7.73 | 10.38 | 13.04 | 15.69 | 18.35 | 21.00 | 28.50 | 36.00 |
| Body weight gain | LPLE | 2.21 | 3.79 | 6.02 | 8.49 | 9.13 | 9.27 | 9.05 | 9.27 | 9.26 |
| (g/kg MBW per d) | LPME | 2.61 | 4.21 | 6.87 | 8.37 | 9.31 | 9.75 | 9.75 | 9.28 | 9.72 |
|  | LPHE | 2.35 | 4.66 | 5.83 | 7.85 | 9.26 | 9.57 | 9.40 | 9.53 | 9.56 |
|  | MPLE | 4.06 | 6.40 | 8.71 | 10.26 | 10.92 | 11.52 | 11.42 | 11.01 | 11.35 |
|  | MPME | 4.86 | 7.02 | 9.14 | 10.69 | 11.53 | 11.40 | 11.59 | 10.61 | 10.99 |
|  | MPHE | 6.43 | 7.99 | 9.54 | 11.25 | 11.19 | 11.34 | 11.72 | 11.22 | 11.14 |
|  | HPLE | 2.97 | 3.69 | 4.79 | 6.41 | 8.06 | 8.84 | 9.77 | 10.56 | 10.41 |
|  | HPME | 2.80 | 3.87 | 5.14 | 6.48 | 7.39 | 9.05 | 9.84 | 11.08 | 11.33 |
|  | HPHE | 2.99 | 4.18 | 5.21 | 6.05 | 7.52 | 8.65 | 9.85 | 10.93 | 10.70 |
| TGC | LPLE | 0.36 | 0.63 | 1.03 | 1.51 | 1.64 | 1.67 | 1.62 | 1.67 | 1.67 |
| (g1/3 per d and per °C) | LPME | 0.43 | 0.70 | 1.19 | 1.48 | 1.68 | 1.77 | 1.77 | 1.67 | 1.76 |
|  | LPHE | 0.38 | 0.78 | 0.99 | 1.38 | 1.66 | 1.73 | 1.70 | 1.72 | 1.73 |
|  | MPLE | 0.67 | 1.08 | 1.52 | 1.82 | 1.95 | 2.08 | 2.05 | 1.97 | 2.04 |
|  | MPME | 0.81 | 1.20 | 1.59 | 1.91 | 2.08 | 2.05 | 2.09 | 1.89 | 1.97 |
|  | MPHE | 1.09 | 1.37 | 1.67 | 2.02 | 2.01 | 2.04 | 2.12 | 2.01 | 2.00 |
|  | HPLE | 0.49 | 0.61 | 0.80 | 1.10 | 1.42 | 1.58 | 1.77 | 1.95 | 1.91 |
|  | HPME | 0.46 | 0.64 | 0.87 | 1.11 | 1.29 | 1.62 | 1.79 | 2.06 | 2.12 |
|  | HPHE | 0.49 | 0.69 | 0.88 | 1.03 | 1.31 | 1.54 | 1.79 | 2.03 | 1.98 |
| FE | LPLE | 0.43 | 0.52 | 0.71 | 0.85 | 0.88 | 0.87 | 0.89 | 0.89 | 0.90 |
| (g/g DM) | LPME | 0.53 | 0.66 | 0.83 | 0.97 | 0.91 | 0.93 | 1.00 | 0.92 | 0.94 |
|  | LPHE | 0.40 | 0.59 | 0.69 | 0.85 | 0.94 | 0.89 | 0.86 | 0.88 | 0.96 |
|  | MPLE | 0.86 | 1.00 | 1.20 | 1.32 | 1.15 | 1.22 | 1.30 | 1.21 | 1.27 |
|  | MPME | 0.89 | 1.05 | 1.17 | 1.31 | 1.43 | 1.31 | 1.31 | 1.34 | 1.32 |
|  | MPHE | 0.90 | 1.08 | 1.22 | 1.36 | 1.15 | 1.25 | 1.33 | 1.25 | 1.25 |
|  | HPLE | 0.68 | 0.84 | 0.92 | 1.07 | 1.19 | 1.21 | 1.31 | 1.31 | 1.37 |
|  | HPME | 0.78 | 1.00 | 1.11 | 1.27 | 1.23 | 1.38 | 1.32 | 1.40 | 1.47 |
|  | HPHE | 0.96 | 1.11 | 1.27 | 1.25 | 1.39 | 1.36 | 1.40 | 1.52 | 1.49 |
| PER | LPLE | 1.57 | 1.90 | 2.59 | 3.10 | 3.23 | 3.19 | 3.27 | 3.26 | 3.32 |
| (g/g) | LPME | 1.88 | 2.36 | 2.99 | 3.48 | 3.30 | 3.38 | 3.64 | 3.35 | 3.45 |
|  | LPHE | 1.41 | 2.09 | 2.46 | 3.02 | 3.35 | 3.18 | 3.08 | 3.21 | 3.53 |
|  | MPLE | 1.95 | 2.29 | 2.76 | 3.05 | 2.67 | 2.84 | 3.04 | 2.85 | 3.00 |
|  | MPME | 2.04 | 2.42 | 2.70 | 3.04 | 3.32 | 3.07 | 3.07 | 3.15 | 3.11 |
|  | MPHE | 2.04 | 2.46 | 2.79 | 3.14 | 2.67 | 2.90 | 3.11 | 2.93 | 2.94 |
|  | HPLE | 1.14 | 1.41 | 1.54 | 1.80 | 2.01 | 2.05 | 2.22 | 2.25 | 2.37 |
|  | HPME | 1.31 | 1.68 | 1.88 | 2.15 | 2.10 | 2.36 | 2.26 | 2.41 | 2.56 |
|  | HPHE | 1.61 | 1.86 | 2.14 | 2.11 | 2.35 | 2.31 | 2.38 | 2.60 | 2.58 |

LP, low-protein diets = 280 g DP/kg DM; MP, medium-protein diets = 440 g DP/kg DM; HP, high-protein diets = 600 g DP/kg DM; LE, low-energy diets = 17 MJ DE/kg DM; ME, medium-energy diets = 19.5 MJ DE/kg DM; HE, high-energy diets = 22 MJ DE/kg DM; MBW, metabolic body weight = ((initial body weight)0.75 + (final body weight)0.75)/2.

\* For the details of procedures and diets, see Tables 1 and 2 and the Material and Methods section. Values are from one aquarium containing initially fifty fry of 0.85 g mean initial body weight. Fish were kept at a temperature of 11.6±0.3°C.

**Supplemental Table 3.** Voluntary feed intake, digestible energy intake, N intake, lysine (Lys) intake, N gain, gross energy (GE) gain and N retention efficiency (NRE; 100 x (N gain/N intake)) of rainbow trout fry fed 81 diets containing three digestible protein (DP) levels and three digestible energy (DE) levels supplemented with nine graded levels of L-lysine (Lys).HCl for 24 (MP diets) or 30 (LP and HP diets) feeding days\*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diet no. |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Dietary Lys concentration (g/kg DM) | LP | 2.26 | 5.38 | 8.51 | 11.63 | 14.75 | 17.88 | 21.00 | 28.50 | 36.00 |
|  | MP | 3.67 | 7.71 | 11.75 | 15.79 | 19.83 | 23.87 | 27.92 | 31.96 | 36.00 |
|  | HP | 5.07 | 7.73 | 10.38 | 13.04 | 15.69 | 18.35 | 21.00 | 28.50 | 36.00 |
| Voluntary feed intake | LPLE | 5.12 | 7.29 | 8.46 | 10.01 | 10.36 | 10.66 | 10.14 | 10.47 | 10.31 |
| (g DM/kg MBW per d) | LPME | 4.97 | 6.39 | 8.24 | 8.65 | 10.18 | 10.45 | 9.72 | 10.12 | 10.37 |
|  | LPHE | 5.87 | 7.84 | 8.42 | 9.25 | 9.89 | 10.80 | 10.99 | 10.82 | 9.97 |
|  | MPLE | 4.75 | 6.41 | 7.26 | 7.77 | 9.50 | 9.47 | 8.78 | 9.07 | 8.92 |
|  | MPME | 5.47 | 6.67 | 7.81 | 8.14 | 8.07 | 8.68 | 8.83 | 7.91 | 8.35 |
|  | MPHE | 7.15 | 7.42 | 7.83 | 8.25 | 9.70 | 9.08 | 8.80 | 8.96 | 8.92 |
|  | HPLE | 3.51 | 3.54 | 4.22 | 4.86 | 5.48 | 5.90 | 6.05 | 6.52 | 6.18 |
|  | HPME | 3.31 | 3.55 | 4.24 | 4.68 | 5.48 | 6.00 | 6.81 | 7.25 | 7.03 |
|  | HPHE | 3.12 | 3.78 | 4.10 | 4.85 | 5.42 | 6.36 | 7.05 | 7.21 | 7.18 |
| Digestible energy intake | LPLE | 86.96 | 123.85 | 143.87 | 170.15 | 176.06 | 181.14 | 172.40 | 178.03 | 175.23 |
| (kJ/kg MBW per d) | LPME | 96.83 | 124.51 | 160.67 | 168.61 | 198.52 | 203.77 | 189.63 | 197.34 | 202.27 |
|  | LPHE | 129.13 | 172.42 | 185.14 | 203.51 | 217.50 | 237.70 | 241.68 | 237.98 | 219.44 |
|  | MPLE | 80.73 | 108.93 | 123.40 | 132.01 | 161.52 | 160.93 | 149.30 | 154.27 | 151.66 |
|  | MPME | 106.57 | 130.03 | 152.21 | 158.78 | 157.45 | 169.21 | 172.19 | 154.33 | 162.87 |
|  | MPHE | 157.27 | 163.19 | 172.29 | 181.55 | 213.34 | 199.81 | 193.61 | 197.23 | 196.33 |
|  | HPLE | 73.69 | 74.39 | 88.62 | 101.95 | 115.06 | 123.80 | 126.94 | 136.93 | 129.60 |
|  | HPME | 70.50 | 75.63 | 90.40 | 99.73 | 116.79 | 127.90 | 145.11 | 154.55 | 149.87 |
|  | HPHE | 68.73 | 83.06 | 90.12 | 106.69 | 119.23 | 139.81 | 155.03 | 158.61 | 157.88 |
| N intake | LPLE | 225.16 | 320.17 | 371.30 | 438.38 | 452.87 | 465.14 | 441.98 | 454.58 | 445.62 |
| (mg/kg MBW per d) | LPME | 222.46 | 285.29 | 367.12 | 384.19 | 451.10 | 461.74 | 428.51 | 442.96 | 450.99 |
|  | LPHE | 267.44 | 355.57 | 380.14 | 416.06 | 442.75 | 481.76 | 487.68 | 475.15 | 433.47 |
|  | MPLE | 332.76 | 447.17 | 504.48 | 537.47 | 654.91 | 649.86 | 600.38 | 617.76 | 604.80 |
|  | MPME | 381.84 | 464.12 | 541.22 | 562.44 | 555.59 | 594.77 | 602.93 | 538.30 | 565.87 |
|  | MPHE | 503.90 | 520.50 | 547.06 | 573.84 | 671.24 | 625.79 | 603.56 | 611.99 | 606.37 |
|  | HPLE | 416.65 | 419.19 | 497.68 | 570.64 | 641.85 | 688.22 | 703.23 | 751.26 | 704.05 |
|  | HPME | 343.23 | 367.22 | 437.78 | 481.67 | 562.57 | 614.46 | 695.28 | 734.92 | 707.27 |
|  | HPHE | 298.09 | 359.27 | 388.76 | 459.00 | 511.56 | 598.26 | 661.59 | 671.64 | 663.39 |
| Lys intake | LPLE | 11.56 | 39.22 | 71.99 | 116.40 | 152.80 | 190.48 | 212.97 | 298.47 | 371.08 |
| (mg/kg MBW per d) | LPME | 11.22 | 34.37 | 70.09 | 100.56 | 150.19 | 186.80 | 204.21 | 288.42 | 373.43 |
|  | LPHE | 13.27 | 42.19 | 71.59 | 107.58 | 145.86 | 193.15 | 230.70 | 308.29 | 359.09 |
|  | MPLE | 17.40 | 49.38 | 85.28 | 122.61 | 188.43 | 226.01 | 245.17 | 290.00 | 321.17 |
|  | MPME | 20.03 | 51.39 | 91.71 | 128.58 | 160.14 | 207.16 | 246.51 | 252.93 | 300.68 |
|  | MPHE | 26.20 | 57.17 | 92.01 | 130.31 | 192.32 | 216.84 | 245.67 | 286.50 | 321.27 |
|  | HPLE | 21.98 | 33.80 | 54.11 | 78.17 | 106.20 | 133.60 | 156.80 | 229.57 | 274.45 |
|  | HPME | 18.33 | 29.96 | 48.12 | 66.66 | 93.97 | 120.32 | 156.27 | 225.87 | 276.68 |
|  | HPHE | 15.84 | 29.16 | 42.52 | 63.21 | 85.03 | 116.58 | 147.99 | 205.47 | 258.36 |
| N gain | LPLE | 19.22 | 52.09 | 99.57 | 171.41 | 182.62 | 188.45 | 169.51 | 188.72 | 191.77 |
| (mg/kg MBW per d) | LPME | 4.47 | 45.59 | 90.33 | 147.23 | 176.16 | 187.70 | 186.78 | 188.06 | 187.70 |
|  | LPHE | 4.00 | 50.61 | 84.35 | 132.95 | 169.81 | 175.36 | 177.31 | 186.47 | 196.41 |
|  | MPLE | 29.01 | 71.72 | 121.96 | 188.23 | 208.18 | 227.02 | 219.41 | 207.31 | 216.80 |
|  | MPME | 36.28 | 77.73 | 141.79 | 180.47 | 210.52 | 214.72 | 220.24 | 152.87 | 212.07 |
|  | MPHE | 57.86 | 91.68 | 138.12 | 174.36 | 203.78 | 207.84 | 230.20 | 211.12 | 218.32 |
|  | HPLE | 37.11 | 44.10 | 81.38 | 115.77 | 154.10 | 186.60 | 221.70 | 240.17 | 244.77 |
|  | HPME | 16.16 | 46.01 | 73.52 | 105.25 | 132.82 | 184.61 | 192.86 | 240.85 | 246.37 |
|  | HPHE | 31.80 | 44.37 | 68.53 | 93.46 | 125.58 | 156.43 | 184.92 | 235.56 | 234.90 |
| GE gain | LPLE | 11.85 | 21.59 | 34.77 | 48.75 | 49.45 | 50.80 | 43.32 | 50.97 | 54.28 |
| (kJ/kg MBW per d) | LPME | 13.66 | 33.28 | 49.41 | 65.04 | 73.08 | 75.23 | 71.80 | 73.22 | 74.58 |
|  | LPHE | 18.01 | 40.90 | 61.28 | 75.59 | 86.07 | 84.77 | 84.39 | 85.12 | 87.32 |
|  | MPLE | 21.22 | 33.71 | 46.49 | 56.11 | 58.03 | 61.20 | 61.58 | 59.31 | 60.50 |
|  | MPME | 25.26 | 41.05 | 56.49 | 67.11 | 76.03 | 74.43 | 76.39 | 64.59 | 74.32 |
|  | MPHE | 39.91 | 50.33 | 66.00 | 79.09 | 79.35 | 81.93 | 82.68 | 77.46 | 81.41 |
|  | HPLE | 11.35 | 17.59 | 27.23 | 37.28 | 47.81 | 53.03 | 57.29 | 60.99 | 60.54 |
|  | HPME | 8.95 | 23.70 | 26.20 | 39.16 | 43.89 | 58.36 | 61.10 | 68.85 | 70.91 |
|  | HPHE | 18.40 | 24.83 | 33.63 | 41.81 | 51.03 | 59.83 | 65.77 | 76.26 | 70.52 |
| NRE | LPLE | 8.54 | 16.27 | 26.82 | 39.10 | 40.32 | 40.51 | 38.35 | 41.52 | 43.03 |
| (%) | LPME | 2.01 | 15.98 | 24.61 | 38.32 | 39.05 | 40.65 | 43.59 | 42.45 | 41.62 |
|  | LPHE | 1.50 | 14.23 | 22.19 | 31.95 | 38.35 | 36.40 | 36.36 | 39.24 | 45.31 |
|  | MPLE | 8.72 | 16.04 | 24.18 | 35.02 | 31.79 | 34.93 | 36.55 | 33.56 | 35.85 |
|  | MPME | 9.50 | 16.75 | 26.20 | 32.09 | 37.89 | 36.10 | 36.53 | 28.40 | 37.48 |
|  | MPHE | 11.48 | 17.61 | 25.25 | 30.38 | 30.36 | 33.21 | 38.14 | 34.50 | 36.00 |
|  | HPLE | 8.91 | 10.52 | 16.35 | 20.29 | 24.01 | 27.11 | 31.53 | 31.97 | 34.77 |
|  | HPME | 4.71 | 12.53 | 16.79 | 21.85 | 23.61 | 30.04 | 27.74 | 32.77 | 34.83 |
|  | HPHE | 10.67 | 12.35 | 17.63 | 20.36 | 24.55 | 26.15 | 27.95 | 35.07 | 35.41 |

LP, low-protein diets = 280 g DP/kg DM; MP, medium-protein diets = 440 g DP/kg DM; HP, high-protein diets = 600 g DP/kg DM; LE, low-energy diets = 17 MJ DE/kg DM; ME, medium-energy diets = 19.5 MJ DE/kg DM; HE, high-energy diets = 22 MJ DE/kg DM; MBW, metabolic body weight = ((initial body weight)0.75 + (final body weight)0.75)/2.

\* For the details of procedures and diets, see Tables 1 and 2 and the Material and Methods section. Values are from one aquarium containing initially fifty fry of 0.85 g mean initial body weight. Fish were kept at a temperature of 11.6±0.3°C.

**Supplemental Table 4**. Whole body composition, expressed in g/100 g fresh body weight for dry matter (DM), crude ash, crude protein and crude lipid or in kJ/100 g fresh body weight for gross energy, of rainbow trout fry fed 81 diets containing three digestible protein (DP) levels and three digestible energy (DE) levels supplemented with nine graded levels of lysine (Lys) for 24 or 30 feeding days\*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Initial  samples | |  |  |  |  | Diet no. |  |  |  |  |  |
|  | Mean | SE |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| DM | 17.72 | 0.060 | LPLE | 18.33 | 19.48 | 20.36 | 20.95 | 20.29 | 20.13 | 19.20 | 20.36 | 20.72 |
|  |  |  | LPME | 17.20 | 21.83 | 21.80 | 24.29 | 24.46 | 24.27 | 24.16 | 24.80 | 24.54 |
|  |  |  | LPHE | 19.36 | 23.61 | 27.00 | 27.30 | 27.43 | 26.55 | 27.06 | 27.39 | 27.76 |
|  | 17.16 | 0.086 | MPLE | 17.23 | 17.82 | 18.52 | 19.46 | 19.63 | 19.71 | 19.71 | 19.68 | 19.64 |
|  |  |  | MPME | 17.11 | 18.67 | 20.27 | 20.83 | 21.72 | 21.74 | 21.92 | 20.04 | 22.16 |
|  |  |  | MPHE | 18.84 | 19.55 | 21.34 | 21.92 | 22.65 | 22.87 | 22.85 | 22.40 | 23.28 |
|  | 17.72 | 0.060 | HPLE | 18.26 | 17.77 | 19.69 | 20.20 | 20.58 | 21.22 | 21.17 | 20.96 | 21.33 |
|  |  |  | HPME | 16.51 | 19.28 | 19.53 | 20.47 | 20.80 | 21.60 | 21.65 | 21.42 | 21.72 |
|  |  |  | HPHE | 19.15 | 19.14 | 20.67 | 21.50 | 21.70 | 22.22 | 21.98 | 22.97 | 22.62 |
| Crude ash | 1.61 | 0.050 | LPLE | 1.93 | 1.88 | 1.94 | 1.98 | 1.86 | 1.86 | 1.73 | 1.94 | 1.91 |
|  |  |  | LPME | 1.51 | 1.79 | 1.58 | 1.84 | 1.82 | 1.81 | 1.80 | 1.85 | 1.83 |
|  |  |  | LPHE | 1.66 | 1.66 | 1.82 | 1.69 | 1.66 | 1.67 | 1.68 | 1.76 | 1.89 |
|  | 1.76 | 0.016 | MPLE | 1.80 | 1.73 | 1.73 | 1.50 | 1.74 | 1.68 | 1.60 | 1.71 | 1.68 |
|  |  |  | MPME | 1.74 | 1.70 | 1.71 | 1.63 | 1.51 | 1.58 | 1.56 | 1.63 | 1.54 |
|  |  |  | MPHE | 1.74 | 1.66 | 1.61 | 1.47 | 1.54 | 1.46 | 1.42 | 1.55 | 1.55 |
|  | 1.61 | 0.050 | HPLE | 1.87 | 1.84 | 1.96 | 1.97 | 1.83 | 1.87 | 1.81 | 1.61 | 1.67 |
|  |  |  | HPME | 1.52 | 1.80 | 1.78 | 1.83 | 1.77 | 1.66 | 1.59 | 1.48 | 1.41 |
|  |  |  | HPHE | 1.89 | 1.64 | 1.69 | 1.76 | 1.63 | 1.57 | 1.47 | 1.40 | 1.37 |
| Crude protein | 12.12 | 0.015 | LPLE | 10.04 | 10.48 | 11.02 | 12.49 | 12.40 | 12.56 | 11.81 | 12.58 | 12.75 |
|  |  |  | LPME | 8.21 | 9.46 | 9.53 | 11.30 | 11.90 | 12.05 | 12.00 | 12.53 | 12.08 |
|  |  |  | LPHE | 8.50 | 9.29 | 10.25 | 11.03 | 11.62 | 11.60 | 11.86 | 12.20 | 12.67 |
|  | 11.96 | 0.017 | MPLE | 8.86 | 9.16 | 9.84 | 11.61 | 11.93 | 12.23 | 12.00 | 11.82 | 11.95 |
|  |  |  | MPME | 8.53 | 8.98 | 10.41 | 10.93 | 11.54 | 11.82 | 11.90 | 9.80 | 12.03 |
|  |  |  | MPHE | 8.40 | 8.91 | 9.93 | 10.26 | 11.53 | 11.57 | 12.20 | 11.81 | 12.17 |
|  | 12.12 | 0.015 | HPLE | 10.44 | 9.99 | 11.31 | 11.58 | 12.00 | 12.92 | 13.73 | 13.80 | 14.17 |
|  |  |  | HPME | 8.94 | 9.92 | 10.32 | 10.85 | 11.51 | 12.59 | 12.22 | 13.32 | 13.33 |
|  |  |  | HPHE | 9.97 | 9.40 | 9.90 | 10.59 | 10.95 | 11.51 | 11.82 | 13.22 | 13.41 |
| Crude lipid§ | 3.99 | 0.005 | LPLE | 6.36 | 7.12 | 7.40 | 6.48 | 6.02 | 5.72 | 5.65 | 5.85 | 6.06 |
|  |  |  | LPME | 7.48 | 10.59 | 10.68 | 11.14 | 10.74 | 10.41 | 10.36 | 10.42 | 10.63 |
|  |  |  | LPHE | 9.20 | 12.66 | 14.93 | 14.58 | 14.14 | 13.28 | 13.52 | 13.42 | 13.20 |
|  | 3.93 | 0.119 | MPLE | 6.57 | 6.93 | 6.96 | 6.35 | 5.96 | 5.81 | 6.11 | 6.15 | 6.02 |
|  |  |  | MPME | 6.88 | 7.99 | 8.16 | 8.28 | 8.67 | 8.35 | 8.46 | 8.61 | 8.58 |
|  |  |  | MPHE | 8.70 | 8.98 | 9.80 | 10.20 | 9.57 | 9.83 | 9.22 | 9.04 | 9.56 |
|  | 3.99 | 0.005 | HPLE | 5.95 | 5.93 | 6.42 | 6.65 | 6.76 | 6.43 | 5.63 | 5.56 | 5.49 |
|  |  |  | HPME | 6.05 | 7.57 | 7.43 | 7.79 | 7.52 | 7.35 | 7.84 | 6.62 | 6.98 |
|  |  |  | HPHE | 7.29 | 8.10 | 9.08 | 9.15 | 9.12 | 9.14 | 8.70 | 8.36 | 7.83 |
| Gross energy|| | 429.29 | 15.38 | LPLE | 462.42 | 494.16 | 521.14 | 535.95 | 514.27 | 519.62 | 466.72 | 521.16 | 548.85 |
|  |  |  | LPME | 462.54 | 609.16 | 621.68 | 683.18 | 701.19 | 695.59 | 668.12 | 703.59 | 691.89 |
|  |  |  | LPHE | 539.11 | 667.82 | 806.69 | 807.60 | 810.78 | 781.76 | 788.46 | 787.12 | 802.94 |
|  | 398.53 | 4.31 | MPLE | 449.58 | 470.85 | 487.84 | 505.88 | 497.83 | 499.07 | 505.46 | 502.77 | 500.56 |
|  |  |  | MPME | 455.48 | 508.44 | 549.04 | 566.15 | 596.48 | 590.11 | 596.61 | 552.40 | 604.50 |
|  |  |  | MPHE | 523.16 | 546.33 | 603.18 | 626.46 | 631.80 | 643.24 | 633.93 | 617.52 | 646.52 |
|  | 429.29 | 15.38 | HPLE | 410.99 | 450.69 | 504.71 | 526.51 | 546.75 | 556.92 | 551.63 | 548.08 | 550.79 |
|  |  |  | HPME | 388.25 | 515.59 | 474.52 | 541.89 | 542.64 | 591.83 | 579.07 | 586.28 | 591.04 |
|  |  |  | HPHE | 502.03 | 511.06 | 552.21 | 591.44 | 602.39 | 623.35 | 615.67 | 647.39 | 614.44 |

LP, low-protein diets = 280 g DP/kg DM; MP, medium-protein diets = 440 g DP/kg DM; HP, high-protein diets = 600 g DP/kg DM; LE, low-energy diets = 17 MJ DE/kg DM; ME, medium-energy diets = 19.5 MJ DE/kg DM; HE, high-energy diets = 22 MJ DE/kg DM.

\* For the details of procedures and diets, see Tables 1 and 2 and the Material and Methods section. Values are from one aquarium containing initially fifty fry. Fish were kept at a temperature of 11.6±0.3°C.

§ Crude lipid was estimated from the following relationship: Crude lipid = 100 – crude ash (% DM) – crude protein (% DM).

|| For MP fed fry, gross energy was estimated from the following relationship: GE = 1.0046\*(23.6\*Crude protein + 39.5\*Crude lipid) – 21.347.

**Supplemental Table 5.** Parameters estimated by fitting the experimental data to a four parameter logistic equation at each protein and energy levels for the response criteria of thermal growth coefficient (TGC; 1000 x ((final body weight)1/3 – (initial body weight)1/3)/(feeding days x temperature), N gain (mg/kg metabolic body weight (MBW) per d), gross energy gain (GE gain; kJ/kg MBW per d), voluntary feed intake (VFI; g DM/kg MBW per d), digestible energy intake (DEI; kJ/kg MBW per d), digestible N intake (DNI; mg/kg MBW per d) and N retention efficiency (NRE; 100 x (N gain/N intake); %)\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Four parameter logistic model parameters | | | | |  |
| Lysine (Lys) requirement in terms of | Criteria | Diets | Rmax | b | c | d | 95%Rmax |  |
| Dietary Lys level | TGC | LPLE | 1.67 c | 0.32 | 49.59 | 0.62 | 13.50 d |
| (g/kg DM) |  | LPME | 1.74 c | 0.35 | 30.87 | 0.65 | 13.71 d |
|  |  | LPHE | 1.74 c | 0.36 | 27.71 | 0.69 | 15.27 c,d |
|  |  | MPLE | 2.03 a,b | 0.52 | 21.02 | 0.74 | 17.34 c |
|  |  | MPME | 2.06 a,b | 0.70 | 32.76 | 0.70 | 16.12 c,d |
|  |  | MPHE | 2.05 a,b | 1.01 | 38.39 | 0.69 | 15.00 c,d |
|  |  | HPLE | 1.94 b | 0.38 | 46.65 | 0.75 | 22.91 b |
|  |  | HPME | 2.14 a | 0.31 | 21.64 | 0.81 | 26.80 a |
|  |  | HPHE | 2.04 a,b | 0.43 | 37.86 | 0.78 | 25.41 a,b |
|  | N gain | LPLE | 188.47 d | -0.28 | 14.19 | 0.69 | 14.92 c |
|  |  | LPME | 190.24 d | -6.37 | 16.37 | 0.70 | 16.25 c |
|  |  | LPHE | 189.54 d | -4.38 | 11.48 | 0.75 | 17.91 b,c |
|  |  | MPLE | 220.48 c | 2.31 | 14.36 | 0.77 | 20.50 b |
|  |  | MPME | 218.25 c | 8.85 | 13.53 | 0.76 | 19.69 b |
|  |  | MPHE | 222.38 b,c | 9.56 | 5.33 | 0.82 | 19.66 b,c |
|  |  | HPLE | 251.07 a | 1.51 | 16.02 | 0.81 | 26.47 a |
|  |  | HPME | 252.76 a | -4.52 | 14.43 | 0.83 | 28.73 a |
|  |  | HPHE | 247.18 a,b | 6.52 | 15.89 | 0.83 | 30.53 a |
|  | GE gain | LPLE | 50.24 e | 7.52 | 35.51 | 0.61 | 12.25 d |
|  |  | LPME | 74.16 b,c | 7.47 | 15.81 | 0.68 | 13.36 c,d |
|  |  | LPHE | 86.11 a | 8.47 | 11.02 | 0.69 | 12.87 d |
|  |  | MPLE | 60.64 d | 15.15 | 16.36 | 0.74 | 16.59 c |
|  |  | MPME | 75.91 b,c | 15.62 | 14.54 | 0.75 | 16.83 b,c,d |
|  |  | MPHE | 81.02 a,b | 26.62 | 24.89 | 0.71 | 14.27 c,d |
|  |  | HPLE | 60.91 d | 6.07 | 31.65 | 0.75 | 21.37 a,b |
|  |  | HPME | 70.92 c | 4.31 | 24.14 | 0.79 | 24.71 a |
|  |  | HPHE | 73.88 b,c | 12.57 | 29.81 | 0.78 | 23.93 a |
|  | VFI | LPLE | 10.40 a | 4.65 | 26.74 | 0.63 | 10.23 c |
|  |  | LPME | 10.14 a | 4.72 | 37.96 | 0.64 | 11.94 c |
|  |  | LPHE | 10.51 a | 5.73 | 35.96 | 0.65 | 11.57 b,c |
|  |  | MPLE | 9.09 b | 4.47 | 41.09 | 0.71 | 15.74 a,b,c |
|  |  | MPME | 8.45 b,c | 4.80 | 40.06 | 0.65 | 11.63 b,c |
|  |  | MPHE | 8.97 b | 7.10 | 53.15 | 0.75 | 15.90 b,c |
|  |  | HPLE | 7.81 c,d | 3.36 | 45.00 | 0.74 | 18.83 b |
|  |  | HPME | 7.88 c,d | 3.38 | 57.88 | 0.76 | 22.68 a |
|  |  | HPHE | 7.29 d | 3.01 | 61.61 | 0.75 | 21.99 a,b |
|  | DEI | LPLE | 177.38 c | 72.18 | 8.95 | 0.68 | 9.86 c |
|  |  | LPME | 199.23 b | 82.45 | 9.65 | 0.72 | 11.67 b,c |
|  |  | LPHE | 231.65 a | 110.26 | 7.74 | 0.72 | 10.16 b,c |
|  |  | MPLE | 155.14 d | 64.27 | 9.36 | 0.77 | 14.61 a,b,c |
|  |  | MPME | 165.92 c | 85.64 | 8.19 | 0.74 | 10.56 b,c |
|  |  | MPHE | 198.26 b | 151.31 | 12.23 | 0.81 | 11.63 b,c |
|  |  | HPLE | 133.72 e | 42.18 | 11.35 | 0.79 | 18.77 a,b |
|  |  | HPME | 155.89 d | 56.13 | 14.14 | 0.82 | 23.82 a |
|  |  | HPHE | 162.97 c,d | 53.84 | 13.44 | 0.81 | 23.07 a |
|  | DNI | LPLE | 452.55 d | 202.26 | 32.21 | 0.62 | 9.91 d |
|  |  | LPME | 444.79 d | 212.75 | 51.54 | 0.62 | 11.50 d |
|  |  | LPHE | 461.52 d | 257.33 | 55.18 | 0.60 | 10.45 c,d |
|  |  | MPLE | 617.63 b,c | 303.98 | 58.82 | 0.68 | 16.64 a,b,c,d |
|  |  | MPME | 577.24 c | 342.49 | 59.13 | 0.62 | 11.10 d |
|  |  | MPHE | 611.74 c | 496.13 | 78.34 | 0.72 | 14.66 c,d |
|  |  | HPLE | 724.05 a | 323.52 | 62.91 | 0.72 | 17.91 b,c |
|  |  | HPME | 724.30 a | 326.33 | 79.48 | 0.74 | 21.86 a |
|  |  | HPHE | 675.99 a,b | 292.44 | 82.40 | 0.74 | 21.37 a,b |
| Dietary Lys level | N gain | LPLE | 188.58 c | -0.44 | 14.10 | 0.40 | 5.98 a,b |
| (g/16 g N) |  | LPME | 190.28 c | -6.52 | 16.34 | 0.42 | 6.50 a |
|  |  | LPHE | 189.44 c | -4.39 | 11.69 | 0.48 | 7.15 a |
|  |  | MPLE | 220.66 b | 0.23 | 14.82 | 0.30 | 4.59 c,d |
|  |  | MPME | 218.73 b | 5.59 | 14.05 | 0.29 | 4.42 d |
|  |  | MPHE | 220.81 b | 8.77 | 6.38 | 0.40 | 4.52 c,d |
|  |  | HPLE | 250.98 a | 1.78 | 16.12 | 0.31 | 4.81 b,c,d |
|  |  | HPME | 253.03 a | -4.54 | 14.19 | 0.35 | 5.25 b,c |
|  |  | HPHE | 247.31 a | 6.58 | 15.79 | 0.37 | 5.56 b |
| Lys intake | N gain | LPLE | 186.35 d | 6.64 | 12.21 | 0.96 | 133.49 b |
| (mg/kg MBW per d) |  | LPME | 188.52 d | 0.33 | 9.28 | 0.97 | 143.76 b |
|  |  | LPHE | 187.27 d | -2.18 | 6.97 | 0.97 | 161.39 a,b |
|  |  | MPLE | 217.81 b | 8.57 | 10.20 | 0.97 | 154.25 a,b |
|  |  | MPME | 218.03 b | 12.27 | 7.72 | 0.97 | 155.15 a,b |
|  |  | MPHE | 221.31 b,c | 8.94 | 2.79 | 0.98 | 137.07 a,b |
|  |  | HPLE | 249.24 a | 9.35 | 5.80 | 0.98 | 186.30 a |
|  |  | HPME | 252.78 a,b | -1.27 | 3.19 | 0.98 | 165.92 a,b |
|  |  | HPHE | 255.30 a,c | 8.38 | 2.52 | 0.98 | 174.32 a,b |
|  | NRE | LPLE | 41.07 a | 6.69 | 11.59 | 0.96 | 119.09 a |
|  |  | LPME | 41.85 a,b | 0.31 | 7.34 | 0.96 | 110.04 a |
|  |  | LPHE | 37.81 a,b,c | -0.79 | 7.73 | 0.96 | 122.65 a |
|  |  | MPLE | 34.89 c | 5.69 | 11.35 | 0.96 | 124.26 a |
|  |  | MPME | 37.24 b,c | 6.89 | 10.43 | 0.97 | 142.27 a |
|  |  | MPHE | 34.10 c | 6.69 | 7.16 | 0.97 | 130.57 a |
|  |  | HPLE | 34.33 c | 5.27 | 4.75 | 0.98 | 145.48 a |
|  |  | HPME | 32.02 c | 1.63 | 5.45 | 0.97 | 104.51 a |
|  |  | HPHE | 36.52 a,b,c | 8.53 | 3.75 | 0.98 | 137.81a |

LP, low-protein diets = 280 g digestible protein (DP)/kg DM; MP, medium-protein diets = 440 g DP/kg DM; HP, high-protein diets = 600 g DP/kg DM; LE, low-energy diets = 17 MJ digestible energy (DE)/kg DM; ME, medium-energy diets = 19.5 MJ DE/kg DM; HE, high-energy diets = 22 MJ DE/kg DM; MBW, metabolic body weight = ((initial body weight)0.75 + (final body weight)0.75)/2; Rmax, plateau value; b, y-intercept; c, shaping parameter that locates the inflection point; d, scaling parameter; 95%Rmax, requirement estimate.

a,b,c,d,e Values within a column for a same response criterion with no common superscript letter are significantly different (0 ∉ 95%CI).

\* For the details of procedures and diets, see Tables 1 and 2 and the Materials and Methods section. Values are from one aquarium containing initially fifty fry of 0.85 g mean initial body weight. Fish were kept at a temperature of 11.6±0.3°C.